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Post-harvest storage effects on guayule latex, rubber, and resin contents and yields

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ABSTRACT

Guayule is a new crop being commercialized for hypoallergenic latex production. Because natural processes that occur in the plant following harvest, notably dehydration, result in rapid loss of latex and immediate processing of guayule shrub for latex on a commercial scale is not feasible, storage conditions that maintain latex concentration and yield need to be established. The objective of this study was to determine the effects of different storage conditions on the extractable latex, total rubber, resin, and guayulin A and B contents, and extractable latex, total rubber, and resin yields in harvested guayule shrub. The experiment was established using plants transplanted into the field at the University of Arizona Maricopa Agricultural Center, Maricopa, AZ, USA, on 22 March 2001. A randomized complete block design with four replications was used. Two germplasm lines (11591 and AZ-2) were used for this experiment. Twenty plants of each line were harvested six times (November 2002, March 2003, July 2003, November 2003, March 2004, and July 2004) from each field plot. Two plants of each line were randomly assigned to each of 10 storage treatment combinations reflecting wet, dry, or wet alternated with dry conditions prior to chipping for latex extraction. Extractable latex content, total rubber content, resin content, and guayulin A and B contents were determined after storage and compared with freshly harvested shrub. Plant biomass, latex yield, rubber yield, and resin yield were also determined and compared with fresh harvested shrub. AZ-2 was significantly lower in latex, rubber, and guayulin A content than 11591, and significantly higher in biomass, latex yield, rubber yield, resin content, resin yield, and guayulin B content. The results from this study show that moist storage of harvested shrub prior to dry chipping allows a higher yield of latex. Storing the shrub under moist conditions may allow more flexible harvesting and processing schedules, by limiting post-harvest latex losses and increasing the time interval between harvesting and processing.

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1. Introduction

Currently the United States imports more than one million metric tons of natural rubber at a cost of about one billion US dollars (Mooibroek and Cornish, 2000). Allergies to Hevea [*Hevea brasiliensis* (A. Juss.) Muell.-Arg.] latex in the United States and Europe have become a serious health problem, especially with health care workers and patients who undergo multiple surgeries (Ownby et al., 1994). Guayule (*Parthenium argentatum* Gray) latex has been shown to be a potential source of hypoallergenic latex for the manufacture of medical and other latex products (Siler and Cornish, 1994; Siler and Cornish, 1995; Siler et al., 1996). Guayule and Hevea are the only plants that are currently grown commercially for natural rubber.

The genus *Parthenium*, a member of the family Asteraceae, is native to most of North America (Whitworth and Whitehead, 1991; Foster and Coffelt, 2005; Ray et al., 2005). The most promising species within *Parthenium* for commercialization as a natural rubber crop is *Parthenium argentatum* or guayule. The other 16 species of *Parthenium* do not produce significant quantities of high quality rubber and most are non-rubber producers. Guayule is a perennial shrub native to the Chihuahuan Desert of northern Mexico and the Big Bend area of Texas. The use of guayule as a natural rubber source dates before 1500 A.D. when Native Americans used its latex to make balls for games.

Guayule has been evaluated in the U.S. as a potential commercial rubber crop during at least three periods (Whitworth and Whitehead, 1991; Foster and Coffelt, 2005; Ray et al., 2005). The first period (1900–1930) was to produce rubber tires for automobiles and bicycles and ended with the great depression. The second period (1940–1945) was to produce rubber for military vehicles during World War II when rubber supplies from Southeast Asia were cut off. The third period (1970–1980) was to produce high performance tires for the military when rubber supplies were short due to high petroleum prices. The current commercialization effort differs from previous efforts in that the primary product is latex rather than solid rubber.

Production and harvesting practices for guayule have been developed previously for bulk/solid rubber uses (Foster and Coffelt, 2005), especially for tires. One of these practices was to field-cure the shrub for 10 to 45 days prior to processing, which dehydrated the shrub, thus reducing the weight of shrub material to be transported, and also maximized the amount of solid rubber recovered by the milling process (Taylor and Chubb, 1952). However, this is an unacceptable practice when harvesting guayule for latex because natural processes that occur in the plant following harvest, most notably dehydration, result in a rapid loss of extractable latex (Cornish et al., 2000; McMahan et al., 2006). Previous work has shown that long-term storage under high temperatures can adversely affect rubber and latex yield and quality (Black et al., 1986; Schloman et al., 1986; Estilai and Hamerstrand, 1989; Dierig et al., 1990; Nakayama and Coates, 1996; Cornish et al., 2000). Two studies noted genotypic differences among the lines used, indicating that improvements in storage behavior may be possible using plant breeding and selection (Estilai and Hamerstrand, 1989; Dierig et al., 1990).

Because immediate processing of guayule shrub for latex on a commercial scale is not feasible, storage conditions that maintain latex concentration and yield need to be established (McMahan et al., 2006). The objective of this study was to determine the effects of different storage conditions on the extractable latex concentration and yield, the total rubber concentration and yield, total resin concentration and yield, and the concentrations of guayulins A and B in harvested guayule shrub.

2. Materials and methods

2.1. *Field design and germplasm lines*

Two germplasm lines (11591 and AZ-2) were used for this experiment. The line 11591 (PI 478640) is an older line that has been grown for many years and is used as a check in our breeding and agronomic studies. AZ-2 (PI 5999675) is a publicly released, improved germplasm line selected for larger plant size and higher rubber and resin yields (Ray et al., 1999).

The experiment was established by transplanting plants in the field at the University of Arizona Maricopa Agricultural Center, Maricopa, AZ, USA, on 22 March 2001. A randomized complete block design with four replications was used. Twenty plants of each line were harvested six times (November 2002, March 2003, July 2003, November 2003, March 2004, and July 2004) from each field plot. Plants were harvested from the field using the method described by Coffelt and Nakayama (2007). This method involves cutting the plants near (50 mm) the soil surface using an electric saw and using all above ground portions of the plant for extracting latex, rubber, resin, and guayulin A and B.

2.2. *Storage treatments*

The 10 storage treatment combinations consisted of various sets of wetting and drying periods (Table 1). In all treatments two randomly selected harvested plants of each line were placed into individual burlap bags (1 bag per replication and 4 replications per treatment and line).

In 4 treatments (Wet 7, Wet 14, Wet 21, Wet 28), the bags were immersed immediately following harvest in a barrel of water for about 1 min to soak the plant material and the bag. The bags then were placed on tables in the shade and kept moist by a mister system for 7 (Wet 7), 14 (Wet 14), 21 (Wet 21), or 28 (Wet 28) days. The shade consisted of a canvas tent with tables about 0.75 m above the ground. The tent had vents to allow natural air movement. No fans or other means of moving air within the tent was used. Bags were placed on the tables and the mister system was about 0.75 m above the bags. In the March and November harvests the misters ran for 1 min two times per day (08:00 and 17:00 h). For the July harvests, the misters were run three times per day (08:00, 13:00, and 17:00 h) for 2 min each time. This was sufficient to keep the bags and plant material moist to the touch.

In four other treatments, the bags were placed dry in the shade for 7 days (Dry 7), dry 7 days followed by wet for 7 days (D7/W7), dry 14 days followed by wet 7 days (D14/W7), and dry 21 days followed by wet 7 days (D21/W7). The last treatment

Table 1 – Ten storage treatment combinations used to evaluate latex, rubber, resin and guayulin concentrations and latex, rubber, resin and biomass yields for two guayule lines at each of six harvest dates

Treatment code	Treatment
Fresh	Harvested and chipped same morning (control)
Dry 7	Harvested and stored in shade dry for 7 days before chipping
Wet 7	Harvested and stored in shade wet 7 days before chipping
Wet 14	Harvested and stored wet in shade 14 days before chipping
Wet 21	Harvested and stored wet in shade 21 days before chipping
Wet 28	Harvested and stored wet in shade 28 days before chipping
D7/W7	Harvested and stored dry in shade 7 days followed by stored wet in shade 7 days before chipping
D14/W7	Harvested and stored dry in shade 14 days followed by stored wet in shade 7 days before chipping
D21/W7	Harvested and stored dry in shade 21 days followed by stored wet in shade 7 days before chipping
Alt D7/W7	Harvested and stored alternately in shade 7 days dry followed by 7 days wet for a total of 28 days before chipping

was to alternate dry and wet conditions for 7 days for a total of 28 days (Alt D7/W7). For the dry portion of the treatments the bags were placed in the same type of canvas tent used in the wet treatments, but without a mister system. For the wet portion of the treatments the bags were moved to the same tent as the wet treatments for the specified amount of time. Except for the Dry 7 treatment, all bags were in the wet treatment tent prior to chipping.

2.3. *Latex, rubber, resin, and guayulin quantification*

Harvested and stored plants were analyzed after treatment for dry biomass weight, latex concentration and yield, rubber concentration and yield, resin concentration and yield, and guayulin A and B concentration. All experiments were harvested and chipped using the method described by Coffelt and Nakayama (2007), except for the modifications in storage noted above prior to chipping. Plants used in the fresh harvested treatment were placed in dry bags as the other treatments and chipped within 2 h of harvest. In this method, plant material passes through the chipper and is mixed with anti-oxidant solution and reground using Waring blenders to produce homogenate for latex analyses (Coffelt and Nakayama, 2007).

Latex concentration in the homogenate was determined by the method described by Cornish et al. (1999). Latex concentrations were determined on a dry weight basis. Resin and rubber concentrations were determined by a modification of the gravimetric method of Black et al. (1983) as described by Veatch-Blohm et al. (2006). Acetone was used to extract the resin followed by cyclohexane to extract the rubber. Concentrations were determined on a dry weight basis. Yields of latex, rubber, and resin are the product of the respective concentrations multiplied by total biomass/2 plants.

Guayulin A and B concentrations were determined from the dried resin fractions obtained for resin analyses. Dried resin samples were dissolved in absolute ethanol to give a final resin concentration of about 10 mg/ml. After filtering, samples were injected onto a 250 mm × 4.6 mm Microsorb™-MV (Varian, Inc., Walnut Creek, CA) C-18 reverse-phase column with pore size of 5 μm at ambient temperature. The mobile phase was a gradient of degassed acetonitrile and water (both HPLC-grade from EMD Chemicals, Gibbstown, NJ) at 1.5 mL/min. [time, min. (% acetonitrile)]: 0 (80), 15 (80), 18 (100), 25 (100), 30 (80), 36 (80). Peak detection was made with a Hitachi HPLC system with a UV diode array detector at 262 nm. The retention time of guayulin B was about 24 min, and that of guayulin A was about 25 min. Peak areas (area counts) were determined using Hitachi HPLC System Manager Software, version 4.0 (Hitachi, Ltd.). Concentrations were calculated by the following formula:

$$\%\,\text{guayulin A or B} = \frac{\text{AC} \times \text{RF} \times \text{mL EtOH}}{\text{mg resin} \times 100{,}000{,}000}$$

where AC is area counts, RF is response factor; guayulin A is 1.235 ng/1000 AC, guayulin B is 0.867 ng/1000 AC (D. Stumpf, personal communication). The percent guayulin is expressed as g of guayulin per g of resin.

All treatments were compared with the currently recommended procedure of fresh harvesting (fresh). Values reported are based on above ground plant dry weights, including leaves. Data were analyzed by analyses of variance and means separated by Fisher L.S.D. at the $P = 0.05$ level. A 2 (lines) × 10 (treatments) factorial model was used with lines, treatments, and replications considered independent variables and latex concentration and yield, resin concentration and yield, rubber concentration and yield, guayulins A and B concentrations, plant biomass, and harvested plant moisture content considered dependent variables. The two and three order interactions with replications within harvest date were combined as the error term. All analyses were done using SAS 9.1 software (SAS Institute, Cary, NC).

3. Results

Because of latex losses caused by dehydration and exacerbated by high temperatures (Cornish et al., 2000) harvested shrub should be processed as soon as possible to protect latex yield. Unprotected latex will coagulate into solid rubber within the plant or harvested plant material, and then only be extractable as solid rubber using organic solvents. The latest commercial guayule processing facility extracts latex using a wet-mill procedure scaled-up from those described earlier (Cornish, 1996; Cornish et al., 1999). An alternative milling procedure for small plots (Coffelt and Nakayama, 2007) used in this study processes harvested guayule plants immediately or at least within 2 h after field cutting by dry chipping into an anti-oxidant solution to maintain the rubber in the form of latex (the aqueous emulsion of rubber particles).

Table 2 – Mean dry plant biomass (kg) for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	1.74 ab[b]	1.45 ab	1.94 ab	1.45 ab	1.46 abc	3.87 a
Dry 7	1.39 bc	1.25 b	1.70 abc	1.25 b	1.40 abc	1.75 b
Wet 7	1.92 a	1.41 ab	1.56 bc	1.40 ab	1.64 ab	1.70 b
Wet 14	1.60 abc	1.54 ab	1.74 abc	1.54 ab	1.63 ab	1.70 b
Wet 21	1.99 a	1.27 b	1.81 abc	1.27 b	1.59 abc	1.61 b
Wet 28	2.03 a	1.32 ab	1.36 c	1.32 ab	1.34 c	1.69 b
D7/W7	1.28 c	1.78 a	2.12 a	1.78 a	1.67 a	1.85 b
D14/W7	1.78 ab	1.66 ab	1.96 ab	1.66 ab	1.35 c	1.96 b
D21/W7	1.45 bc	1.68 ab	1.65 abc	1.68 ab	1.34 c	2.12 b
Alt D7/W7	1.25 c	1.62 ab	1.63 abc	1.62 ab	1.37 bc	1.82 b
Line						
11591	1.05 b	0.82 b	0.96 b	0.82 b	0.80 b	1.16 b
AZ-2	2.23 a	2.17 a	2.54 a	2.17 a	2.15 a	2.85 a
Interaction						
Treatment × Line[c]	ns	ns	ns	ns	ns	ns

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's L.S.D. at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance *f* test, *significant at the $P \leq 0.05$ level according to analysis of variance *F* test.

Results for the 10 storage treatments showed a significant harvest date by treatment interaction and other interactions were generally not significant, thus the results are presented as averages for the main effects (storage treatment and line) by harvest date (Tables 2–11). Analyses of variance showed significant differences between lines for all traits studied (Tables 2–10). AZ-2 was significantly lower in latex, rubber, and guayulin A concentration than 11591, and significantly higher in biomass, latex yield, rubber yield, resin concentration, resin yield, and guayulin B concentration. The results for rubber and resin concentrations and yields and biomass are similar to previous studies using these lines (Ray et al., 1999). These are the first reports for these lines on latex concentration and yield, and guayulin A and B concentrations.

Plant biomass remained fairly constant across harvest dates for all storage treatments with an average dry weight of about 1.7 kg/two plants (Table 2). This was unexpected, since the plants were expected to increase in biomass with age. No obvious explanation was found for this lack of increase in biomass. Results from this study are in contrast to previous reports (Tingey and Foote, 1947; Nakayama, 1991; Milthorpe

Table 3 – Mean latex concentration (%) for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	0.70 c[b]	1.13 d	1.47 d	1.33 e	2.46 def	1.54 bc
Dry 7	0.25 e	0.38 e	0.13 f	0.45 f	0.83 g	0.12 e
Wet 7	0.55 cde	2.15 c	0.87 e	2.40 cd	3.02 bcd	1.72 bc
Wet 14	1.06 b	2.43 bc	3.40 a	2.56 bc	3.54 ab	2.24 a
Wet 21	1.70 a	3.22 a	2.91 b	3.22 a	3.71 a	1.84 ab
Wet 28	0.62 cd	3.42 a	2.15 c	2.92 ab	2.86 cde	1.31 c
D7/W7	0.36 de	1.18 d	0.24 f	0.79 f	2.76 de	1.70 bc
D14/W7	0.47 cde	1.27 d	0.07 f	0.71 f	2.29 ef	0.76 d
D21/W7	0.34 de	1.24 d	0.04 f	0.80 f	1.89 f	0.13 e
Alt D7/W7	1.86 a	2.55 b	0.27 f	2.06 d	3.39 abc	0.52 de
Line						
11591	0.89 a	2.26 a	1.42 a	2.05 a	2.99 a	1.23 a
AZ-2	0.69 b	1.54 b	0.89 b	1.39 b	2.36 b	1.15 a
Interaction						
Treatment × Line[c]	*	*	*	*	ns	ns

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's LSD at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance *f* test, *significant at the $P \leq 0.05$ level according to analysis of variance *F* test.

Table 4 – Mean latex yield (g) per two plants for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	10.65 de[b]	15.27 d	25.40 b	17.03 c	34.81 de	59.49 a
Dry 7	3.75 g	4.36 e	1.98 cd	5.19 d	11.01 g	2.00 e
Wet 7	9.26 de	27.01 bc	11.90 c	32.22 ab	46.17 abc	27.98 bc
Wet 14	16.83 c	34.81 ab	53.34 a	35.52 ab	52.33 ab	36.25 b
Wet 21	29.01 a	37.45 a	46.11 a	37.50 a	54.72 a	29.42 bc
Wet 28	12.00 d	40.26 a	27.61 b	36.91 ab	38.63 cde	24.75 c
D7/W7	3.96 fg	19.00 cd	3.51 cd	11.66 cd	40.46 cde	29.02 bc
D14/W7	7.60 ef	19.24 cd	1.43 d	9.93 d	30.32 ef	13.19 d
D21/W7	5.01 fg	20.46 cd	0.72 d	10.94 cd	22.74 f	2.31 de
Alt D7/W7	21.03 b	37.13 a	3.51 cd	30.29 b	43.15 bcd	6.09 de
Line						
11591	9.29 b	18.38 b	12.91 b	16.83 b	24.52 b	14.75 b
AZ-2	14.45 a	32.62 a	22.19 a	28.61 a	50.35 a	31.35 a
Interaction						
Treatment × Line[c]	*	ns	*	*	ns	*

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's LSD at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance *f* test, *significant at the $P \leq 0.05$ level according to analysis of variance *F* test.

et al., 1994) that showed higher biomass in the fall. There were significant differences among the storage treatments for biomass each of the six harvest dates, but no consistent differences were found for a particular treatment or type of treatment (wet vs. dry or wet or dry vs. fresh). The line by storage treatment interaction for biomass was not significant for any of the harvest dates, indicating both lines responded similarly to the storage treatments for biomass.

Extractable latex concentration was greatest for the March harvest dates (Table 3). This was especially true for the dry treatments. Significant differences were found among storage treatments at all harvest dates. Generally the four wet storage treatments (Wet 7, Wet 14, Wet 21, and Wet 28) were higher in extractable latex concentration than the fresh and other storage treatments. The Wet 14 and Wet 21 storage treatments were consistently the highest in extractable latex concentration. Interestingly the storage treatment which alternated 7 days dry and 7 days wet (Alt D7/W7) was high in extractable latex concentration for the two November and two March harvests, but not for the two July harvests. The line by storage treatment interaction was significant for four of the harvest dates (November 2002 and 2003, March 2003, and July

Table 5 – Mean rubber concentration (%) for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	3.07 d[b]	4.82 cd	4.56 d	5.10 c	5.58 c	4.70 f
Dry 7	3.13 cd	4.36 d	4.75 cd	5.49 abc	5.70 c	4.90 ef
Wet 7	3.31 bcd	5.56 ab	4.81 cd	5.64 abc	6.36 b	5.43 cde
Wet 14	3.76 a	5.12 bc	5.97 a	5.72 ab	6.51 ab	6.03 ab
Wet 21	3.71 a	5.19 abc	5.76 a	5.90 a	6.94 a	5.88 abc
Wet 28	3.56 abc	5.60 ab	6.11 a	5.85 ab	6.90 a	6.26 a
D7/W7	3.52 abc	5.06 bc	5.16 bc	5.64 abc	6.25 b	5.60 bcd
D14/W7	3.47 abcd	5.76 a	5.66 ab	5.87 a	6.62 ab	5.38 cde
D21/W7	3.61 ab	5.64 ab	5.13 bc	5.25 bc	6.64 ab	5.15 def
Alt D7/W7	3.63 ab	5.80 a	6.06 a	5.99 a	6.66 ab	5.90 abc
Line						
11591	4.14 a	6.20 a	6.34 a	6.50 a	7.55 a	6.22 a
AZ-2	2.81 b	4.38 b	4.45 b	4.79 b	5.28 b	4.83 b
Interaction						
Treatment × Line[c]	ns	ns	ns	ns	ns	*

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's LSD at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance *f* test, *significant at the $P \leq 0.05$ level according to analysis of variance *F* test.

Table 6 – Mean rubber yield (g) per two plants for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	47.2 bcd[b]	64.6 bc	81.7 abc	66.7 c	77.4 cd	177.7 a
Dry 7	41.1 d	51.7 c	75.3 bc	63.2 c	74.7 d	79.7 b
Wet 7	57.7 ab	73.5 abc	70.6 c	75.0 abc	96.4 ab	89.6 b
Wet 14	56.9 ab	70.4 abc	96.6 abc	81.8 abc	95.8 abc	98.5 b
Wet 21	68.3 a	62.8 bc	95.9 abc	71.4 bc	100.4 a	90.9 b
Wet 28	67.6 a	67.9 abc	78.0 abc	72.7 abc	84.6 abcd	99.1 b
D7/W7	41.9 d	82.4 ab	99.1 ab	95.6 a	95.9 abc	97.1 b
D14/W7	54.5 bc	88.2 ab	102.1 a	87.1 abc	82.8 abcd	100.2 b
D21/W7	47.5 bcd	87.4 ab	77.5 abc	80.9 abc	80.6 bcd	99.6 b
Alt D7/W7	42.8 cd	90.7 a	88.6 abc	92.9 ab	81.8 abcd	89.0 b
Line						
11591	43.5 b	51.2 b	60.4 b	53.8 b	60.6 b	70.2 b
AZ-2	61.6 a	96.7 a	112.7 a	103.6 a	113.5 a	134.0 a
Interaction						
Treatment × Line[c]	ns	ns	ns	ns	ns	ns

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's LSD at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance f test, *significant at the $P \leq 0.05$ level according to analysis of variance F test.

2003). The interaction appeared to be due to the fact that the difference between lines was less under the dry storage treatments than the wet storage treatments even though 11591 was always higher than AZ-2 in extractable latex concentration.

Latex yield results (Table 4) were generally the same as those for extractable latex concentration with the four wet storage treatments greater in latex yield than the fresh harvested treatment, except for the last harvest date. The March harvests had greater latex yields than the November harvest preceding and the July harvests following them. This was especially noticeable in the Dry treatments. The Dry 7 storage treatment was significantly less in latex yield than the fresh harvested treatment and most of the other storage treatments for all harvest dates. The storage treatment which alternated 7 days dry and 7 days wet (Alt D7/W7) was high in latex yield for the two November and two March harvests, but not for the two July harvests. This is not unexpected since the latex concentration for this storage treatment was high for these same harvest dates. The line by storage treatment interaction was significant for four of the harvest dates (November 2002 and 2003 and July 2003 and 2004). The interaction appeared to be due to the fact that the difference between lines was less

Table 7 – Mean resin concentration (%) for 10 storage treatments averaged for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main Factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	6.76 ab[b]	8.54 ab	7.11 b	8.29 ab	9.50 bc	8.30 bcd
Dry 7	6.73 ab	8.26 b	7.61 b	8.65 ab	9.42 c	8.19 cd
Wet 7	6.97 ab	8.63 ab	7.26 b	8.05 b	9.78 bc	8.11 d
Wet 14	7.39 a	9.12 ab	7.27 b	8.13 b	9.57 bc	9.05 abc
Wet 21	7.24 ab	9.54 a	7.64 b	8.55 ab	9.97 abc	8.70 bcd
Wet 28	7.23 ab	9.25 ab	8.95 a	8.53 ab	9.79 bc	9.73 a
D7/W7	7.07 ab	8.95 ab	7.55 b	8.80 ab	9.83 bc	8.55 bcd
D14/W7	6.38 b	9.26 ab	8.15 ab	8.65 ab	9.99 abc	9.16 ab
D21/W7	6.95 ab	9.58 a	8.04 ab	9.02 a	10.61 a	8.27 cd
Alt D7/W7	6.63 ab	8.71 ab	7.90 ab	8.86 ab	10.20 ab	8.68 bcd
Line						
11591	6.28 b	8.19 b	6.95 b	7.46 b	8.64 b	7.30 b
AZ-2	7.59 a	9.78 a	8.54 a	9.65 a	11.09 a	10.04 a
Interaction						
Treatment × Line[c]	ns	ns	ns	ns	ns	ns

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's LSD at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance f test, *significant at the $P \leq 0.05$ level according to analysis of variance F test.

Table 8 – Mean resin yield (g) per two plants for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main Factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	121.0 abc[b]	126.3 ab	142.8 ab	124.7 ab	148.0 abc	334.4 a
Dry 7	93.2 c	105.2 b	136.1 ab	113.0 b	139.1 bc	154.8 b
Wet 7	137.1 ab	127.8 ab	119.6 b	120.8 ab	166.6 ab	150.4 b
Wet 14	125.5 abc	147.5 ab	130.6 ab	132.4 ab	162.6 abc	164.5 b
Wet 21	147.9 ab	126.7 ab	145.6 ab	113.1 b	165.7 abc	148.3 b
Wet 28	150.4 a	123.8 ab	129.8 ab	120.5 ab	138.5 c	165.5 b
D7/W7	95.3 c	169.2 a	163.7 ab	169.6 a	168.7 a	169.3 b
D14/W7	115.7 bc	158.0 a	171.8 a	154.7 ab	143.3 abc	186.6 b
D21/W7	99.0 c	162.5 a	138.1 ab	162.6 ab	149.1 abc	180.9 b
Alt D7/W7	91.2 c	148.0 ab	138.5 ab	152.4 ab	148.8 abc	167.9 b
Line						
11591	66.3 b	67.1 b	66.9 b	61.6 b	68.8 b	84.0 b
AZ-2	169.0 a	211.9 a	216.4 a	211.2 a	237.3 a	280.5 a
Interaction						
Treatment × Line[c]	ns	ns	ns	ns	ns	ns

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's LSD at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance *f* test, *significant at the $P \leq 0.05$ level according to analysis of variance *F* test.

under the dry storage treatments than the wet storage treatments even though AZ-2 was always higher than 11591 in latex yield.

Rubber concentration and yield increased for the same month of harvest in the second year compared to the first year of the study, especially the November harvests (Tables 5 and 6). As with the extractable latex concentration and yield, the March harvests tended to be higher than the November harvest preceding and the July harvests following them. No consistent storage treatment effects were found on rubber concentration or yield, although the fresh harvested treatment and the Dry 7 storage treatment tended to be the lowest in rubber concentration and yield for all harvest dates. The line by storage treatment interaction was not significant for rubber concentration or yield at any of the harvest dates, except for rubber concentration at the last harvest date. Results from this study are similar to previous reports (Tingey and Foote, 1947; Nakayama, 1991; Milthorpe et al., 1994) that showed higher rubber content in the spring.

Results for resin concentration and yield were similar to those for rubber concentration and yield (Tables 7 and 8). Resin concentrations were greater at the March harvest dates

Table 9 – Mean guayulin A concentration (g per g resin) for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main Factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	0.17 b[b]	1.56 bc	2.04 f	3.34 d	2.91 c	3.21 d
Dry 7	0.47 a	1.84 ab	1.51 f	3.29 d	2.95 c	3.89 d
Wet 7	0.50 a	2.06 ab	2.42 f	7.62 a	2.76 c	7.93 bc
Wet 14	0.40 ab	1.78 ab	4.28 de	8.34 a	2.76 cd	10.82 a
Wet 21	0.51 a	1.86 ab	6.09 bc	7.77 a	6.25 a	10.50 a
Wet 28	0.37 ab	1.91 ab	7.50 a	4.82 bc	6.43 a	10.72 a
D7/W7	0.34 ab	1.27 c	4.00 e	5.80 b	2.16 d	8.06 bc
D14/W7	0.41 a	2.27 a	7.97 a	3.93 cd	5.47 b	7.47 c
D21/W7	0.34 ab	1.81 ab	7.19 ab	2.94 de	4.88 b	5.03 d
Alt D7/W7	0.39 ab	1.78 ab	5.41 cd	2.17 e	6.40 a	9.60 ab
Line						
11591	0.60 a	2.85 a	8.07 a	8.23 a	7.05 a	11.54 a
AZ-2	0.18 b	0.77 b	1.61 b	1.77 b	1.55 b	3.91 b
Interaction						
Treatment × Line[c]	*	ns	*	*	*	*

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's LSD at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance *f* test, *significant at the $P \leq 0.05$ level according to analysis of variance *F* test.

Table 10 – Mean guayulin B concentration (g per g of resin) for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main Factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	0.028 abc[b]	0.14 bcd	0.21 cd	0.33 de	0.24 d	0.36 de
Dry 7	0.033 abc	0.16 ab	0.13 d	0.28 de	0.23 d	0.76 bc
Wet 7	0.043 ab	0.20 a	0.20 d	0.56 b	0.21 d	0.30 e
Wet 14	0.044 a	0.11 cd	0.29 bc	0.68 a	0.21 d	0.94 a
Wet 21	0.031 abc	0.15 bc	0.38 b	0.63 ab	0.52 a	0.88 ab
Wet 28	0.038 abc	0.18 ab	0.50 a	0.37 cd	0.49 ab	0.97 a
D7/W7	0.036 abc	0.10 d	0.30 b	0.46 c	0.18 d	0.65 c
D14/W7	0.022 c	0.17 ab	0.53 a	0.30 de	0.41 c	0.65 c
D21/W7	0.025 bc	0.17 ab	0.53 a	0.25 e	0.44 bc	0.44 d
Alt D7/W7	0.036 abc	0.13 bcd	0.37 b	0.13 f	0.45 bc	0.69 c
Line						
11591	0.022 b	0.11 b	0.29 b	0.31 b	0.25 b	0.40 b
AZ-2	0.046 a	0.19 a	0.41 a	0.48 a	0.43 a	0.93 a
Interaction						
Treatment × Line[c]	*	ns	ns	ns	*	*

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's LSD at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance f test, *significant at the $P \leq 0.05$ level according to analysis of variance F test.

than the summer (July) or fall (November) harvest dates. No consistent storage treatment effects were found on resin concentration or resin yield. The line by storage treatment interaction was not significant for any of the harvest dates for either resin concentration or resin yield.

No storage treatment effects were found on guayulin A concentration at the first three harvest dates (Table 9). At the last three harvest dates the wet storage treatments (Wet 7, Wet 14, Wet 21, and Wet 28) were generally higher in guayulin A than the other storage treatments. Although the line by storage treatment interaction was significant for five of the harvest dates, no line by treatment combination could be found to account for the interaction.

The guayulin B concentrations were not consistently effected by storage treatment over harvest dates (Table 10). The concentrations for guayulin B were much lower than those for guayulin A (Tables 9 and 10). Lines that tend to be higher in one of the guayulins tend to be lower in the other, such as in this experiment where 11591 was higher in guayulin A and AZ-2 was higher in guayulin B. This same trend was not true for storage treatment effects on guayulin concentrations. The line by storage treatment interaction was significant for

Table 11 – Mean moisture content (%) for two main factors (10 storage treatments and two guayule lines) and significance of the storage treatment × line interaction for each of six harvest dates

Main Factor	November 2002	March 2003	July 2003	November 2003	March 2004	July 2004
Treatment[a]						
Fresh	64.96 d[b]	54.88 d	66.77 bc	54.88 d	51.59 e	71.40 d
Dry 7	26.30 e	27.21 f	11.84 c	27.21 f	24.12 f	15.39 e
Wet 7	96.09 c	101.11 b	55.94 bc	101.11 b	100.85 b	148.81 ab
Wet 14	139.10 ab	97.62 b	258.23 a	97.62 b	98.00 b	158.69 a
Wet 21	127.60 b	133.33 a	127.32 abc	133.31 a	106.91 ab	153.71 ab
Wet 28	103 35 c	123.32 a	170.75 ab	123.32 a	117.37 a	140.88 b
D7/W7	105.18 c	42.10 e	60.99 bc	42.10 e	66.50 cd	98.93 c
D14/W7	96.33 c	68.05 c	64.20 bc	68.05 c	73.37 c	74.90 d
D21/W7	95.99 c	54.56 de	71.61 bc	54.56 de	59.78 de	76.61 d
Alt D7/W7	151.39 a	78.45 c	78.61 bc	78.45 c	74.24 c	77.68 d
Line						
11591	106.65 a	80.38 a	121.49 a	80.38 a	83.96 a	110.14 a
AZ-2	94.61 b	75.74 a	71.76 a	75.74 a	70.58 b	93.26 b
Interaction						
Treatment × Line[c]	ns	ns	ns	ns	ns	ns

[a] Treatment codes are given in Table 1.
[b] Means followed by the same letter within columns and main factor are not significantly different according to Fisher's LSD at $P = 0.05$.
[c] ns: not significant ($P > 0.05$) according to analysis of variance f test, *significant at the $P \leq 0.05$ level according to analysis of variance F test.

three of the six harvest dates, but no consistent line by treatment combination(s) could be identified as the cause for the interaction. These are the first reports of guayulin A and B concentration for these lines and storage treatments.

4. Discussion

Interactions between lines and harvest date were generally not significant, indicating similar results could be expected for both lines at each harvest date. Interactions between lines and treatments were generally not significant, indicating that both lines responded similarly to the 10 storage treatments tested. This is in contrast to previous reports (Estilai and Hamerstrand, 1989; Dierig et al., 1990) that reported significant interactions between lines and method of storage.

Moist storage treatments often resulted in higher extractable latex concentration and latex yield over freshly harvested shrub (Tables 3 and 4). Treatments without initial moisture treatment (dry) were generally lower than freshly harvested shrub in extractable latex concentration and yield. Results from a study to evaluate latex quality from a subsample of these same treatments also showed that latex quality was maintained under the wet storage conditions, but polymer molecular weight was reduced up to 30% under extended dry storage times (McMahan et al., 2006).

Three possible hypotheses are proposed for the increases in extractable latex concentration and yield observed in this study. The first hypothesis is that the harvested plants continued to make rubber/latex after they were harvested, such as some plants do in an "after ripening process" with other traits. If this is true, then the rubber yields and concentrations should also be higher for the wet treatments than the fresh harvested and dry treatments. The results (Tables 5 and 6) do not support this hypothesis, because rubber concentrations and yields did not increase significantly for the wet treatments as did the latex concentrations and yields. In addition, a ready supply of assimilate to support additional biosynthesis during storage appears to be lacking.

A second hypothesis is that the biomass varied among treatments. This could be due to larger plants being inadvertently selected for the wet treatments or by decaying leaf material during the wet storage being lost resulting in mostly stem biomass being left to chip. The first condition would result in higher yields for the wet treatments because of increased biomass. The second condition would result in higher extractable latex concentrations and yields, since leaves do not contain significant amounts of latex. If either of these is true, then the biomass for the wet treatments should be significantly different from the biomass for the dry treatments. Because biomass did not change consistently or significantly for treatments (Table 2), then this hypothesis must not be correct. In addition, we were careful to make sure leaf material was not lost prior to the chipping process when handling all storage treatments. Thus, the type and amount of shrub material used in all treatments was similar.

A third hypothesis is that the increases in latex yield were due to increases in extractable latex and not increased biomass or increased total rubber concentration. The results (Tables 3 and 4) support this hypothesis, because the same wet storage treatments that were higher in extractable latex concentration were higher in latex yield than the other storage treatments. One reason the wet treatments may have been higher in extractable latex concentration is the moisture content of the shrub at harvest. Cornish et al. (2000) proposed that by the time guayule branches have lost half of their moisture all of the latex has coagulated into solid rubber. Moisture data (db) of the plants used in this study show that the samples with the highest moisture content were the wet treatments and these were generally the storage treatments with the highest extractable latex concentration (Tables 3 and 11).

The higher extractable latex content in the wet treatments suggests that the wet treatments contained enough moisture to prevent the individual rubber particles from being exposed to dehydration from the time of harvest through the chipping process until the chipped plant material contacted the anti-oxidant solution. The dehydration of individual rubber particles can be either by direct contact with the air or by their particular parenchyma cells being too close to the air to be protected from water loss.

If rubber was not in the form of extractable latex in the fresh shrub at harvest time, then the total rubber content should be different between the storage treatments. However, the total rubber content was not different among treatments (Table 5) indicating the wet storage treatments were high in extractable latex at harvest.

Some evidence also exists to suggest that more than plant moisture per se may be involved in the high extractable latex concentrations. For example, all storage treatments except the Dry 7 for all harvest dates and the Dry 7/Wet 7 storage treatment for the March 2003 and November 2003 harvest dates were above the moisture level proposed by Cornish et al. (2000) as the minimum needed for optimum latex extraction. Thus, all treatments should have similar extractable latex concentrations, unless higher moisture levels are needed to maintain maximum extractable latex concentrations or some other factors are occurring in the Wet treatments.

The low extractable latex levels in the dry treatments could be explained by the initial dry period of 7 days resulting in conversion of the latex to solid rubber, but it does not explain why some of these treatments then have significantly higher extractable latex concentrations than the Dry 7 storage treatment. If the latex is irreversibly converted to solid rubber, then all the dry treatments should have the same extractable latex concentrations. Some of these dry then rewet storage treatments have extractable latex concentrations equal to the fresh treatment. This is especially true for the alternating dry 7/wet 7 storage treatment which had extractable latex levels equal to the wet storage treatments for the March and November harvest dates.

Another indication that something more than moisture content per se may be involved in some of these storage treatments is the differences among the wet storage treatments. The Wet 14 and Wet 21 storage treatments are significantly higher than the Wet 7 and Wet 28 storage treatments in extractable latex concentration for five of the six harvest dates. It may be that during this two to three week storage period there is some structural changes in the plant cell walls or other plant cell components which makes latex extraction easier or that protects the latex from destruction during the extraction process. Determining what this factor(s) is was beyond the scope of the current study.

More research is needed to establish post-harvest storage parameters that best protect the latex in shrub post-harvest and to identify what the factor(s) is that results in higher extractable latex contents for the Wet 14 and Wet 21 storage treatments. The results from this study show that a moist pretreatment of harvested shrub protected the latex fraction during dry chipping prior to subsequent wet-grind and quantification processes. Storing harvested shrub under moist conditions may allow industry more flexible harvesting and processing schedules by extending the time period between harvesting and latex extraction processes without significant latex loss.

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